

CLAIMS

1. A cell population comprising insulin-producing cells derived from human embryonic stem cells.
2. The cell population of claim 1 enriched for insulin-producing cells derived from human embryonic stem cells.
3. The cell population of claim 2 wherein the enrichment comprises treatment of the human embryonic stem cells with insulin, transferrin and selenite.
4. The cell population of claim 1 comprising selected insulin-producing cells derived from human embryonic stem cells.
5. The cell population of claim 1 comprising isolated insulin-producing cells derived from human embryonic stem cells.
6. The cell population of claim 1 comprising cloned insulin-producing cells derived from human embryonic stem cells.
7. A cell population comprising regulatable insulin-producing cells derived from human embryonic stem cells.
8. The cell population of claim 7 comprising glucose-responsive insulin-producing cells derived from human embryonic stem cells.
9. The cell population of claim 8 enriched for glucose-responsive insulin-producing cells derived from human embryonic stem cells.
10. The cell population of claim 9 wherein the enrichment comprises treatment with insulin, transferrin and selenite.
11. The cell population of claim 8 comprising selected glucose-responsive insulin-producing cells derived from human embryonic stem cells.
12. The cell population of claim 8 comprising isolated glucose responsive insulin-producing cells derived from human embryonic stem cells.

13. The cell population of claim 8 comprising cloned glucose-responsive insulin-producing cells derived from human embryonic stem cells.
14. The glucose responsive insulin-producing cells of claim 8
wherein said cells express at least one gene from the group of: insulin, islet
5 glucokinase, Glut-2 glucose transporter, Glut-1 glucose transporter, insulin
promoter factor1/pancreatic and duodenal homeobox gene 1 IPF1/PDX1
transcription factor, and Ngn3 transcription factor.
15. A cell population comprising stable insulin-producing cells derived from
human embryonic stem cells.
- 10 16. The cell population of claim 15 comprising stable clonal insulin-producing cells
derived from human embryonic stem cells.
17. The cell population of claim 15 comprising insulin-producing cells derived from
human embryonic stem cells overexpressing hTERT.
18. The cell population of claim 15 comprising insulin-producing cells derived from
15 human embryonic stem cells stably transfected with a construct comprising an
insulin promoter.
19. The cell population of claim 18 comprising cloned insulin-producing cells
derived from human embryonic stem cells stably transfected with an insulin
promoter.
- 20 20. A clone of non-differentiated human stem cells stably transfected with a vector
comprising the DNA coding sequence of human insulin promoter.
21. A cell population comprising pluripotent precursors of beta islet cells of the
pancreas derived from human embryonic stem cells, stably transfected with an
insulin promoter.

22. A cell population comprising committed precursors of beta islet cells of the pancreas derived from human embryonic stem cells.
23. The cells clone of claim 20, wherein a reporter gene is fused downstream of the insulin promoter sequence.
- 5 24. The cells clone of claim 20, wherein the expression of the reporter gene is regulated by the insulin-promoter gene.
25. The cells clone of claim 20, comprising insulin-producing cells.
26. A method for in vitro enrichment of insulin-producing cells derived from stem cells, comprising the steps of:
- 10 (i) culturing undifferentiated pluripotent stem cells in a chemically defined serum-free culture medium complemented with supplements selected from: serum replacement; nonessential amino acids; mercaptoethanol; glutamine; or fibroblast growth factor; and
- 15 (ii) disaggregating and transferring the adherent cell cultures from (i) to suspension culture in bacterial-grade petri dish; and
- (iii) adding to the culture medium of the cells from (ii) supplements selected from the group consisting of: insulin; transferrin and sodium selenite (ITS); glucose; nicotinamide; keratinocyte growth factor; fibroblast growth factor; vascular endothelial growth factor; epidermal growth factor; nerve growth factor; activin; and β -cellulin.
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27. The method in claim 26 comprising the following step:
- (i) culturing undifferentiated pluripotent stem cells on a feeder layer in a chemically defined serum-free culture medium complemented
- 25 with supplements selected from: serum replacement; nonessential

- amino acids; mercaptoethanol; glutamine; or fibroblast growth factor, and
- 5 (ii) disaggregating and transferring the adherent cell cultures from (i) to suspension culture in bacterial-grade petri dish; and
- (iii) culturing the cells in (ii) for 4-5 in a culture medium as in (i) in the absence of fibroblast growth factor; and
- (iv) disaggregating and transferring the embryoid bodies formed in (iii) to fibronectin coated tissue culture dishes in serum-free medium;
- 10 (v) adding to the culture medium of (iv) supplements selected from the group consisting of: fibronectin; transferrin and sodium selenite (ITS); and
- (vi) adding to the culture medium of (v) supplements selected from the group consisting of: B27 supplement (GIBCO); N2 supplement (GIBCO); laminin; and fibroblast growth factor; and
- 15 (vii) replacing the culture medium in (vi) with culture medium comprising supplements selected from the group consisting of: B27 supplement (GIBCO); N2 supplement (GIBCO); laminin; and nicotinamide.
- 20 28. In a method of cell replacement therapy, the improvement which comprises administering to a subject in need of such therapy insulin producing cells derived from human embryonic stem cells.
29. The method of claim 28 wherein the cells are transplanted into the subject's pancreas.
30. The method of claim 28 wherein the cells are transplanted into an ectopic site in the subject.

31. A method of treating a patient in need thereof with insulin producing cells derived from human embryonic stem cells comprising transplantation of a cell population comprising insulin producing cells derived from human embryonic stem cells.

5 **32.** The method of claim 31 comprising transplantation of a cell population comprising insulin producing cells derived from human embryonic stem cells into the pancreas.

33. The method of claim 31 comprising transplantation of a cell population comprising insulin producing cells derived from human embryonic stem cells to
10 an ectopic site.